# Expert Opinion on Investigational Drugs

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**Review**



# PSMA specific antibodies and their diagnostic and therapeutic use

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Prostate-specific membrane antigen (PSMA) is a membrane-bound glycoprotein highly restricted to prostatic epithelial cells. PSMA expression is increased in association with prostatic cancer, particularly in hormone refractory disease. Given its membrane-bound character, PSMA is an ideal sentinel molecule for use in targeting prostatic cancer cells. Monoclonal antibodies specific for PSMA are available, beginning with the antibody 7E11.C5 which originally defined PSMA and which has been developed for use in cancer detection *via* immunoscintiscanning in the ProstaScint™ test. Newer second generation antibodies specific for both linear amino acid sequence epitopes and protein conformational epitopes on the extracellular domain of PSMA have been reported. Although most of these are murine antibodies, both humanised and fully human examples have been developed. These antibodies are beginning to work their way into clinical applications for potential improved diagnostic and therapeutic uses. Results to date suggest that antibodies specific for extracellular epitopes are significantly better for clinical uses *in vivo* than the 7E11.C5 antibody that is specific for an intracellular epitope. Current knowledge relating to PSMA-specific antibodies and their clinical uses and potential is described and evaluated.

***Keywords:*** *antibody therapy, linear sequence epitopes, monoclonal antibodies, prostate cancer, prostate-specific membrane antigen, PSMA, protein conformational epitopes*

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## 1. Introduction

PSMA is a highly glycosylated 750 amino acid type II transmembrane protein expressed on the surface of prostatic epithelial cells. It is composed of three distinct domains, a 19 amino acid intracellular, N-terminal domain, a 24 amino acid transmembrane domain and a 707 amino acid extracellular domain [1]. Although the amino acid composition of PSMA indicates it has a protein molecular weight of approximately 84,000, PSMA migrates in an SDS gel with an apparent molecular weight of 110 - 120 kDa through use of ten potential N-linked glycosylation sites which occur in the extracellular domain of the protein [2].

PSMA was identified using the monoclonal antibody 7E11.C5 [3] and has been shown to be highly prostate specific [3-9]. Quantitative expression data indicates that, at protein level, significant PSMA expression is confined to the prostate [10]. Weak extraprostatic expression of PSMA has been detected in other human tissues such as brain, salivary gland and small intestine tissues by genetic, immunohistochemical and Western blot results

[3-10]. Other results demonstrate that PSMA expression is increased in prostatic cancer, particularly in association with disease progression and in hormone refractory tumours [4,11,12].

High expression of PSMA in normal and malignant prostatic tissues and its nature as an integral membrane protein are properties which make PSMA particularly useful for antibody targeting in cell-based diagnostic or therapeutic strategies. This especially applies to antibodies directed against the extracellular domain of the protein. Multiple investigators have reported the isolation of second generation antibodies specific for extracellular epitopes on PSMA. The purpose of this review is to summarise what is known about these antibodies, their current clinical utility and discuss which properties are optimally suited for *in vivo* clinical applications in humans.

## 2. PSMA specific monoclonal antibodies

### 2.1 7E11.C5

The 7E11.C5 antibody was developed in 1987 by Horoszewicz *et al.* by immunising mice with human prostatic cancer LNCaP cells [3]. Resulting hybridomas were screened for their reactivity with LNCaP cells and negative reactivity with 32 other human non-prostatic normal and malignant cell lines. Immunohistochemical results reported by Horoszewicz *et al.* demonstrated nearly complete 7E11.C5 antibody binding specificity to epithelial cells of the human prostate [3]. This staining pattern has been largely confirmed in subsequent studies with weak extraprostatic expression of PSMA observed in brain, salivary gland and small intestine tissues [6,8]. PSMA, as defined by the 7E11.C5 antibody, has been shown to be a superior immunohistochemical marker than prostate-specific antigen (PSA) for prostatic epithelial cells [13,14]. In addition, staining of vascular endothelium of non-prostatic tumours has been reported using the 7E11.C5 antibody [8]. As discussed below, this result has been confirmed in other studies which conclude that PSMA expression is characteristic of tumour-associated neovasculature but is absent in normal vascular endothelia [15,16].

The high specificity of the 7E11.C5 antibody for prostatic epithelial cells and the overexpression of PSMA in prostatic cancer formed the basis for use of this antibody in the ProstaScint™ test for *in vivo* diagnostic imaging of occult prostate cancer. Studies have demonstrated that the 7E11.C5 antibody is specific for a linear protein sequence epitope composed of the first six amino acids from the N-terminal of the PSMA protein. This portion of the protein is distributed on the cytoplasmic side of the plasma membrane [17]. This places the epitope in an isolated environment in live prostatic epithelial cells. Thus, the 7E11.C5 antibody is most probably detecting non-viable or apoptotic cells. To accumulate an adequate quantity of such cells or cellular debris in tumours for detection, a minimum tumour size is likely to be required. This physical restriction is likely to limit the potential of this antibody for *in vivo* diagnostic or therapeutic applications and most probably also limits the clinical performance of the ProstaScint™ imaging agent.

### 2.2 Antipeptide antibodies

The identification of the intracellular distribution of the 7E11.C5 antibody epitope has helped focus efforts by multiple groups in generating monoclonal antibodies specific for extracellular epitopes on PSMA. Antibody production efforts have utilised full length protein, fragments of the PSMA protein and short peptides composed of 6 - 8 amino acids of the PSMA sequence selected for their probability of comprising an antigenic determinant by use of, for example, the Hopp and Woods test [18].

Murphy *et al.* reported the first monoclonal antibody specific for an extracellular epitope on PSMA [19]. This IgM antibody, 3F5.4G6, was obtained by immunising mice with a peptide corresponding to aa716-723 of the PSMA protein which is distributed near the C-terminal of the protein. Results demonstrated that the 3F5.4G6 antibody was capable of immunoprecipitating native PSMA from an LNCaP cell detergent lysate. The immunoprecipitated protein was reactive with the PSMA-specific 7E11.C5 antibody in a subsequent Western blot. The immunoreactivity of this peptide region was further demonstrated in another report in which animals were immunised with a crude membrane preparation from a prostatic carcinoma and an antibody, designated 4G5, was screened out using the aa716-723 peptide [20]. This result also confirms that the aa716-723 peptide epitope is exposed and accessible in the native protein and its conformation in the native protein is most probably similar to that found in the isolated peptide.

Another application of this approach has involved generation of rabbit polyclonal antiserum using other

potential antigenic determinants within PSMA (Holmes, unpublished results). For example, rabbits immunised with KLH-peptide conjugates with aa63-68, aa132-137, or aa482-487 peptides in each case generated high antipeptide specific immunoreactivities. However, no binding to full length PSMA could be demonstrated with these antisera under conditions that gave saturating antipeptide reactivity. Presumably, expression of these epitopes in the context of the protein was important and affected the antibody binding ability. This observation may be a property of antibodies to these protein regions, or perhaps can be overcome by the use of slightly longer eight amino acid peptides as was utilised by Murphy *et al.* [19].

### 2.3. Linear epitope specific antibodies

Multiple groups have reported other anti-PSMA monoclonal antibodies specific for linear amino acid sequence epitopes distributed throughout the extracellular domain [10,15,21,27-30]. In general, these antibodies have been derived by immunisation of animals with full length PSMA either in membranes or as purified protein. Thus, although the epitopes recognised by these antibodies are of the same type as those recognised by antipeptide antibodies, their location in the protein sequence is not precisely known.

Liu *et al.* reported the isolation of four hybridomas, each secreting an antibody specific for the extracellular domain of PSMA [21]. Competitive binding studies demonstrated that the four antibodies defined two distinct, non-competing epitopes on the extracellular domain of PSMA. The antibodies were also shown to bind to viable LNCaP cells *in vitro* and showed strong immunohistochemical staining of prostatic epithelium and vascular endothelium within a wide variety of carcinomas [21]. These antibodies were shown to bind to denatured PSMA based upon their ability to detect PSMA by Western blot [21]. A subsequent report demonstrated that viable LNCaP cells internalise antibodies of this panel and that PSMA is constitutively endocytosed in LNCaP cells, a process whose rate is increased by anti-PSMA antibodies [22]. These observations suggested utility of these antibodies for targeting and delivery of toxins, drugs, or radioisotopes to the interior of prostate cancer cells.

Recent studies of binding properties of these antibodies after radiolabelling indicate competition for binding sites occurs between certain antibody pairs suggesting at least two closely distributed but non-identical binding sites are recognised [23]. In addition, quantification of the number of sites per cell bound by the antibodies, indicated a decrease of about 40%, when comparing the number of sites detected in permeabilised LNCaP cells with viable cells. The data presented demonstrated that efficient antibody uptake by LNCaP cells occurred along with metabolic processing of the antibody. The results demonstrated that elimination of the radioisotope from the cell was dependent on the nature of the radiolabel used. That is, antibody degradation and efficient elimination of $^{131}$I-label was reported, however, radiometal chelates from degraded antibody molecules were released from LNCaP cells at a significantly slower rate suggesting a much greater radiotherapeutic potential for radiometal chelates such as $^{90}$Y-DOTA. In general, the results demonstrated that antibodies J415 and J591 had the highest binding affinity and were promising reagents for targeting PSMA *in vivo* [23].

Studies of immunoconjugates incorporating either cytotoxic drugs [24], $^{90}$Y [25] or the α-particle emitter $^{213}$Bi [26] into the J591 antibody have been conducted to test their ability to specifically kill prostatic cancer LNCaP cell tumours grown in nude mice. Immunoconjugates composed of the maytansine analogue DM1 coupled to the J591 antibody provided a reduction of tumour size in mice with minimal toxicity at a dose of 300 µg/day [24]. Unconjugated DM1 at equimolar doses was lethal and PC3 cell tumours which do not express PSMA were unaffected by the immunoconjugate demonstrating effective antigen-specific delivery of the immunoconjugate to PSMA positive tumours with low systemic toxicity. Smith-Jones *et al.* reported that $^{90}$Y-DOTA-J591 reduced the tumour volume at dose ranges between 30 - 90 µCi [25]. Re-treatment resulted in further reduction in tumour size or complete regression. Uncontrolled tumour growth occurred in mice treated with $^{90}$Y-DOTA-labelled irrelevant mouse antibody [25]. $^{213}$Bi-labelled J591 demonstrated effective LNCaP cell killing both *in vitro* and *in vivo* in LNCaP cell tumour bearing nude mice associated with internalisation of the radiolabelled J591 antibody [26]. Median tumour free survival times were significantly increased for animals receiving $^{213}$Bi-J591 compared with a $^{213}$Bi-labelled irrelevant antibody or unlabelled J591. Serum PSA levels, as secreted from the LNCaP cell tumours, was also decreased in animals receiving $^{213}$Bi-J591 compared with other treatment groups.

Overall, these results suggest a potential for drug and radioimmunoconjugates incorporating antibodies specific for the extracellular domain of PSMA for therapy of prostatic cancer.

Immunohistochemical studies using these and other antibodies have been conducted to confirm PSMA expression in vascular endothelium from a variety of human non-prostatic cancers and absence of expression in normal neovasculature [15,16]. Despite staining of vascular cells in cancerous tumours, no staining of neoplastic cells from non-prostatic origins was observed. Thus, PSMA specific monoclonal antibodies may also be effective in general targeting of tumour neovasculature *in vivo*.

A panel of monoclonal antibodies specific for epitopes distributed within the extracellular domain of PSMA has been reported by Murphy *et al.* [27]. This report detailed binding specificity of monoclonal antibodies reactive with distinct regions of the PSMA extracellular domain selected from a large panel of 36 distinct linear epitope specific monoclonal antibodies [28]. From this large panel of antibodies, certain generalisations can be made. Solid-phase ELISA studies demonstrated that these antibodies bound to native full-length PSMA, as well as to denatured recombinant fusion proteins containing portions of the PSMA molecule. Efficient binding to denatured full-length PSMA by these linear epitope specific antibodies was demonstrated by their ability to detect PSMA in Western blot experiments. Moderate binding of extracellular linear epitope specific antibodies occurred to non-viable PSMA expressing cells when compared with antibody binding to intracellular epitopes by flow cytometric analysis [27]. Staining intensity by flow cytometry was generally weaker with viable cells than with non-viable or fixed cells when linear epitope specific antibodies were used.

Other anti-PSMA antibodies have been produced such as Hybritech PEQ226.5 and PM2J004.5 antibodies [15]. Both antibodies effectively stain PSMA expressed in tissues by immunohistochemistry with the PEQ226.5 antibody recognising an extracellular epitope and PM2J004.5 recognising an intracellular epitope. The PEQ226.5 antibody has been paired with 7E11.C5 in a sandwich ELISA and used to study specificity of PSMA expression at a protein level in human tissues [10]. Very high levels of PSMA were found to be present in normal and malignant prostatic tissues. Low levels of PSMA were observed in membranes from ovary and breast. Negligible levels (about 200- to 500-fold lower than found in prostatic tissues) were found in membranes from skin, liver, large and small intestine and kidney. Thus, at protein level, quantification by sandwich ELISA indicates PSMA to be overwhelmingly prostate-specific.

Olson recently reported the isolation of two murine antibodies specific for linear epitopes in the extracellular domain of PSMA [29]. Although characterisation information is currently limited, one antibody was reported to react with both native and denatured PSMA, the other only with denatured PSMA. Finally, Wolchok reported the production of a panel of murine monoclonal antibodies from a mouse immunised with human PSMA [30]. Antibody specificity was determined by ELISA, Western blot and flow cytometry. Approximately 90% of the ELISA reactive clones recognised both human and mouse PSMA. Mouse PSMA was localised to a subset of proximal renal tubules.

### 2.4 Conformational epitope specific antibodies

A second class of antibodies specific for epitopes present on the extracellular domain of PSMA recognise conformational folding of the PSMA molecule. These epitopes arise from the juxtapositioning of amino acid residues from differing portions of the linear sequence in close proximity in 3-dimensional space in a manner characteristic of the native protein. Such antibodies have inherently dissimilar properties from linear epitope antibodies, most notable is their inability to bind to denatured PSMA. Tino *et al.* [28], reported the isolation of three murine hybridomas which secrete antibodies capable of binding to at least two distinct conformational epitopes on PSMA. Their results demonstrated the inability of these antibodies to bind to fragments of PSMA, or to heat-denatured full length PSMA. Additionally, these antibodies were incapable of detecting PSMA in a Western blot. In contrast, native PSMA was efficiently immunoprecipitated by this antibody panel. Most significantly, flow cytometric analysis demonstrated strong and equivalent immunostaining of both viable and non-viable PSMA expressing cells.

A panel of five fully-human monoclonal antibodies which bind to a similar conformational epitope present in the extracellular domain of PSMA have been developed using HuMab-Mouse™ strains [31]. As with murine conformational antibodies, these human antibodies do not recognise denatured

antigen and demonstrate strong high affinity binding to either viable or fixed PSMA-expressing cells by flow cytometry. Other studies indicate specificity for prostatic epithelial cells in immunohistochemical analyses with human tissues, efficient internalisation into PSMA-expressing cells *in vitro*, capability of mediating a strong ADCC but not CDC response and efficient biodistribution to PSMA-expressing tumours in mouse model systems (Holmes, unpublished results).

It is particularly noteworthy to emphasise that in all cases so far tested, flow cytometric studies of antibodies specific for conformational epitopes of PSMA demonstrate significantly stronger staining of PSMA-expressing cells (often one log or greater staining intensity) compared with that observed with linear epitope-specific antibodies. Binding of linear epitope-specific antibodies to LNCaP cells is sensitive to the viability of the cells with substantially less binding occurring to viable cells (or as measured as a lower number of apparent sites per cell). One possible explanation for this is the presence of an intracellular pool of PSMA that is only available in non-viable or fixed cells. However, no difference in staining intensity is observed with conformational antibodies when either viable cells or non-viable unfixed or fixed cells are used. This suggests perhaps instead that an intracellular PSMA pool is not large and/or that linear epitope antibodies are able to bind to only a portion of the total cell surface PSMA molecules recognised by conformational antibodies. This could be a function of linear sequence epitope crypticity by virtue of protein folding or glycosylation effects. In contrast, protein folding effects define conformational epitopes and thus these antibodies should not be sensitive to epitope crypticity. This is an important distinction and suggests that conformational antibodies will prove to be superior targeting reagents for *in vivo* applications in humans.

Recently, the isolation of four additional murine antibodies specific for conformational epitopes on PSMA has been reported [29]. These antibodies also do not bind denatured PSMA and efficiently bind to PSMA-expressing cells. Future studies will be required to characterise the nature of the conformational epitopes recognised by these antibodies and their detailed binding properties.

## 3. Current clinical applications with PSMA specific antibodies

### 3.1 ProstaScint™ scans

Primary extraprostatic spread or failure after prostate cancer treatment can occur locally at the prostatic fossa and/or metastasise to regional and/or distant lymphatics and possibly bone [32]. Non-invasive diagnostic tools such as computed tomography (CT) or magnetic resonance imaging (MRI) are sensitive to the size of lymph nodes but cannot detect the presence of prostatic cancer cells [33]. Although not effective for bone metastases, one means to directly target lymph node metastases is the ProstaScint™ scan which is based upon use of $^{111}$In-labeled 7E11.C5 antibody [34,35]. This immuno-scintigraphic scan recognises PSMA expression chiefly in lymph nodes and the prostate bed [36].

Multiple investigators have studied the sensitivity and specificity of the ProstaScint™ scan in prostate cancer patients who underwent conventional CT or MRI scanning, surgical or needle biopsies, or rising serum PSA after radical prostatectomy [36-47]. Pooled results from 637 ProstaScint™ scans described in these reports had a sensitivity of 73% and an accuracy of 82%. For comparison, the sensitivity of conventional CT scanning ranges from about 4 - 15% in these patient groups [33,41,47]. This is similar to the 15% sensitivity reported for MRI [47]. The ProstaScint™ scan is also useful in determining prior to treatment if prostate cancer will recur or has spread to other parts of the body [42,43]. Khan *et al.* have reported that salvage radiotherapy was more likely to lead to a durable complete PSA response in men with prostate cancer who failed radical prostatectomy and had a negative ProstaScint™ scan outside the pelvis as compared with those who had a positive scan [43]. ProstaScint™ was also more predictable than prognostic tables in predicting lymph node involvement prior to lymph-adenectomy in 198 prostate cancer patients with clinical stage T2 to T4 [43].

While no staging modality has been ideal in diagnosis of local recurrence, regional or distant soft lymph node metastases of prostate cancer, ProstaScint™ has contributed to diagnosis, complemented and often exceeded the diagnostic ability of conventional modalities [33,36-50]. Given the epitope specificity of the 7E11.C5 monoclonal antibody used in the ProstaScint™ scan [17], it is likely that a superior scan with

enhanced sensitivity that is capable of identifying smaller lesions would be possible through use of a monoclonal antibody specific for an epitope within the extracellular domain of PSMA and which efficiently binds to viable prostate cancer cells.

### 3.2 Clinical studies of *in vivo* antibody targeting and therapy of prostate cancer

A Phase I dose-escalation study has been conducted using $^{90}$Y-labelled CYT-356 (murine antibody 7E11.C5) in patients with hormone-refractory prostate cancer that had first been imaged using $^{111}$In-CYT-356 [51]. Doses of $^{90}$Y ranged from 1.83 - 12 mCi/m$^2$. The maximum tolerated dose of the radioimmunoconjugate was 9 mCi/m$^2$ with myelosuppression as the dose limiting toxicity. No patients (0/12) had a complete or partial response, although 3/12 had a transient subjective improvement in their symptoms. A slightly longer period before progression in patients receiving the highest dose compared with the next lower dose was also observed. These results are consistent with the specificity of the CYT-356 (7E11.C5) antibody for an intracellular epitope of PSMA and its resultant inability to bind to viable prostatic cancer cells. A potential therapeutic effect from $^{90}$Y-CYT-356 would have to come from a bystander effect wherein radiolabelled antibody would bind to non-viable or apoptotic PSMA-expressing cells within the tumour in an effort to treat the adjacent viable tumour cells. This is a severe limitation of the CYT-356 antibody. A much greater likelihood for effective treatment would be expected using antibodies specific for PSMA epitopes expressed on the outer surface of tumour cells allowing direct binding of the therapeutic antibody conjugates to viable cells for cell killing.

Clinical applications involving second-generation antibodies specific for extracellular epitopes on PSMA, although in their early stages, offer promise. The linear epitope specific murine J591 antibody [21] and its humanised counterpart huJ591 [52] are being used in Phase I biodistribution trials. Biodistribution results with the murine J591 antibody are complete and indicate selective localisation to bone or soft tissue disease with no localisation to sites other than prostatic cancer sites [53,54]. Of 32 evaluable patients, overall tumour targeting was reported in 26/32 (81%) patients. Targeting was observed in 25/30 (83%) of patients with bone disease and 8/11 (73%) of patients with soft tissue disease. Preliminary results from a Phase I trial using the huJ591 antibody with trace labelling with $^{111}$In indicate no apparent toxicity or immunogenicity of the humanised antibody [53]. Early targeting results indicate antibody uptake demonstrating targeting with excellent cancer specificity to both bone and soft tissue disease. Additional Phase I trials with $^{90}$Y-labelled huJ591 for treatment are planned.

### 3.3 Use of PSMA as a cellular immunotherapy target

In addition to antibody based applications utilising PSMA as a therapeutic target, active cellular immunotherapy for prostate cancer has been studied utilising antigen-presenting dendritic cells loaded with peptides derived from PSMA to activate a T-cell response [55]. Positive results were achieved in Phase I and II trials with two PSMA HLA-A2-restricted epitopes exogenously loaded onto dendritic cells. Approximately 30% of study participants showed treatment benefit, defined as a steady decrease in serum levels of PSA and/or stabilisation or decrease in metastatic tumour burden [56]. These results were quite encouraging, yet they could not circumvent the conceptual drawback that the immunodominant, cellular targets on the surface of prostate tumour cells are not known. In order to effectively address this concern, a Phase I/II clinical trial is currently underway involving autologous dendritic cells loaded with whole, recombinant human PSMA protein. This approach directs a significant portion of the antigen into the MHC class I processing and presentation pathway, even though it is exogenously added. Such a strategy is aimed at mounting a cellular immune response in hormone-refractory prostate cancer patients and to overcome tolerance to PSMA that, after all, is also expressed on normal prostate cells. Preliminary results suggest that both cellular and humoral responses to PSMA can be induced, regardless of the advanced age and disease state of the participants (Elgamal, unpublished results). Over 50 injections were administered intradermally with no serious adverse events, at doses from 5 - 20 x 10$^6$ cells. Compared with baseline, 9/10 evaluable subjects became positive upon delayed-type hypersensitivity testing against a vaccine component. Seven of nine subjects converted to a seropositive state, as evidenced by the development of significant titres of anti-PSMA antibodies. This also suggested that tolerance was broken, at least in the realm of humoral immunity. Finally, to date, more than half of evaluable patients have displayed positive cellular immune

responses (*via* proliferation assay) to PSMA. In total, these initial data produced promising results. It is hoped that this strategy may provide additional therapeutic means to effectively treat prostate and other cancers.

## 4. Expert opinion and conclusion

The high prostate tissue/tumour specific expression and integral plasma membrane distribution of PSMA creates an ideal combination for its use as an *in vivo* target for antibody-based prostate cancer diagnosis and therapy. Antibody properties also are critically important for optimal results. Most attractive are specific human or humanised monoclonal antibodies that optimally bind to antigen expressed on the extracellular surface of live cells. These include antibodies that recognise both linear amino acid sequence and protein conformational epitopes present on the extracellular portion of PSMA. Linear sequence epitope antibodies are currently in use in human clinical trials and show considerable promise. Similar studies using human conformational antibodies will be conducted. *In vitro* properties of conformational antibodies suggest that they may prove to be optimal targeting reagents for clinical diagnostic and therapeutic applications. Given that few treatment options currently exist for patients with hormone refractory metastatic prostate cancer, antibody-directed therapy offers considerable potential as a stand alone therapy, or in combination with other treatment regimens including dendritic cell based cellular immunotherapy targeting PSMA expressing tumours. Antibody therapy could perhaps have its greatest impact on earlier stage patients in helping prevent recurrence of cancer, particularly in understaged patients. These new therapeutic options should lead to more effective and economical diagnosis and treatment of the disease.

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## Bibliography

Papers of special note have been highlighted as:
- • of interest
- •• of considerable interest


1. ISRAELI RS, POWELL CT, FAIR WR *et al.*: **Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen.** *Cancer Res.* (1993) **3**:227-230.
2. HOLMES EH, GREENE TG, TINO WT *et al.*: **Analysis of glycosylation of prostate specific membrane antigen derived from LNCaP cells, prostatic carcinoma tumors and serum from prostate cancer patients.** *Prostate* (1996) 7(Suppl):25-29.
3. HOROSZEWICZ JS, KAWINSKI E, MURPHY GP: **Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients.** *Anticancer Res.* (1987) 7:927-936.
4. KAWAKAMI M, NAKAYAMA J: **Enhanced expression of prostate-specific membrane antigen gene in prostate cancer as revealed by *in situ* hybridization.** *Cancer Res.* (1997) **57**:2321-2324.
5. TROYER JK, BECKETT ML, WRIGHT GL JR: **Detection and characterization of the prostate-specific membrane antigen (PSMA) in tissue extracts and body fluids.** *Int. J. Cancer* (1995) **62**:552-558.
6. LOPES AD, DAVIS WL, ROSENSTRAUS MJ *et al.*: **Immunohistochemical and Pharmacokinetic Characterization of the Site-specific Immunoconjugate CYT-356 Derived from Antiprostate Monoclonal Antibody 7E11-C5.** *Cancer Res.* (1990) **50**:6423-6429.
7. ROSENSTROUS MJ, DAVIS WL, LOPES AD *et al.*: ***In vitro* and *in vivo* reactivity of anti-prostate monoclonal antibody immunoconjugate 7E11.C5.3-GYK-DTPA.** *Antibody Immunoconj. Radiopharm.* (1990) **3**:54.
8. SILVER DA, PELLICER I, FAIR WR *et al.*: **Prostate-specific membrane antigen expression in normal and malignant human tissues.** *Clin. Cancer Res.* (1997) **3**:81-85.
9. WRIGHT GL JR, GROB M, HALEY C *et al.*: **Upregulation of prostate-specific antigen after androgen deprivation therapy.** *Urology* (1996) **48**:326-334.
10. SOKOLOFF RL, NORTON KC, GASIOR CL, MARKER KM, GRAUER LS: **A dual-monoclonal sandwich assay for prostate-specific membrane antigen: Levels in tissues, seminal fluid and urine.** *Prostate* (2000) **43**:150-157.
11. ISRAELI RS, POWELL CT, CORR JG *et al.*: **Expression of the prostate-specific membrane antigen.** *Cancer Res.* (1994) **54**:1807-1811.
12. CHANG SS, REUTER VE, HESTON WDW, GRAUER LS, GAUDIN PS: **Short term neoadjuvant androgen deprivation therapy does not affect prostate specific**

**membrane antigen expression in prostate tissues.** *Cancer* (2000) **88**:407-415.

13. BOSTWICK DG, PACELLI A, BLUTE M *et al.*: **Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: study of 184 cases.** *Cancer* (1998) **82**:2256-2261.

14. SWEAT SD, PACELLI A, MURPHY GP, BOSTWICK DG: **Prostate specific membrane antigen expression is greatest in prostate adenocarcinoma and lymph node metastases.** *Urology* (1998) **52**:637-640.

15. CHANG SS, REUTER VE, HESTON WDW, BANDER NH, GRAUER LS, GAUDIN PB: **Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature.** *Cancer Res.* (1999) **59**:3192-3198.

16. CHANG SS, O'KEEFE DS, BACIC DJ, REUTER VE, HESTON WDW, GAUDIN PB: **Prostate-specific membrane antigen is produced in tumor-associated neovasculature.** *Clin. Cancer Res.* (1999) **5**:2674-2681.

17. TROYER JK, FENG Q, BECKETT ML *et al.*: **Biochemical characterization and mapping of the 7e11-C5.3 epitope of the prostate-specific membrane antigen.** *Urology. Oncol.* (1995) **1**:29-37.

18. HOPP TP, WOODS KR: **Prediction of protein antigenic determinants from amino acid sequences.** *Proc. Natl. Acad. Sci. USA* (1981) **78**:3824-3828.

19. MURPHY GP, TINO WT, HOLMES EH *et al.*: **Measurement of prostate-specific membrane antigen in the serum with a new antibody.** *Prostate* (1996) **28**:266-271.
- First report of a monoclonal antibody specific for the extracellular domain of PSMA.

20. ZHU ZY, ZHONG CP, XU WF *et al.*: **PSMA mimotope isolated from phage displayed peptide library can induce PSMA specific immune response.** *Cell Res.* (1999) **9**:271-280.

21. LIU H, MOY P, KIM S *et al.*: **Monoclonal antibodies to the extracellular domain of prostate-specific membrane antigen also react with tumor vascular endothelium.** *Cancer Res.* (1997) **57**:3629-3634.
- Description of PSMA extracellular domain-specific antibodies recognising linear sequence epitopes.

22. LIU H, RAJASEKARAN AK, MOY P *et al.*: **Constitutive and antibody-induced internalization of prostate-specific membrane antigen.** *Cancer Res.* (1998) **58**:4055-4060.

23. SMITH-JONES PM, VALLABAHAJOSULA S, GOLDSMITH SJ *et al.*: ***In vitro* characterization of radiolabeled monoclonal antibodies specific for the extracellular domain of prostate-specific membrane antigen.** *Cancer Res.* (2000) **60**:5237-5243.
- Description of antibody binding properties to prostatic cancer cells.

24. RICHSTONE L, YAO D, KIM S, LIU H, CHARI RJ, BANDER NH: **Eradication of prostate cancer xenografts by immunoconjugates targeting the extracellular domain of prostate-specific membrane antigen ($PSMA_{exD}$).** *Proc. Amer. Soc. Clin. Oncol.* (2000) **19**:338a.

25. SMITH-JONES PM, VALLABHAJOSULA S, NAVARRO V, GOLDSMITH SJ, BANDER NH: **$^{90}$Y-huJ591 Mab specific to PSMA: Radioimmunotherapy (RIT) studies in nude mice with prostate cancer LNCaP tumor.** *Eur. J. Nucl. Med.* (2000) **27**:S951.

26. MCDEVITT MR, BARENDSWAARD E, MA D *et al.*: **An α-particle emitting antibody ([$^{213}$Bi]J591) for radioimmunotherapy of prostate cancer.** *Cancer Res.* (2000) **60**:6095-6100.

27. MURPHY GP, GREENE TG, TINO WT, BOYNTON AL, HOLMES EH: **Isolation and characterization of monoclonal antibodies specific for the extracellular domain of prostate-specific membrane antigen.** *J. Urol.* (1998) **160**:2396-2401.
- Description of PSMA extracellular domain-specific antibodies recognising linear sequence epitopes.

28. TINO WT, HUBER MJ, LAKE TP, GREENE TG, MURPHY GP, HOLMES EH: **Isolation and characterization of monoclonal antibodies specific for protein conformational epitopes present in prostate-specific membrane antigen (PSMA).** *Hybridoma* (2000) **19**:249-257.
- Description of PSMA extracellular domain-specific antibodies recognising protein conformational epitopes.

29. OLSON W: **Development of vaccine and antibody therapies targeting prostate-specific membrane antigen.** *7th Annual CaP CURE Scientific Retreat* (2000) Lake Tahoe, NV, USA. www.capcure.org

30. WOLCHOK JD: **Xenogeneic genetic immunization with prostate-specific membrane antigen cDNAs.** *7th Annual CaP CURE Scientific Retreat* (2000) Lake Tahoe, NV, USA. www.capcure.org:34

31. HOLMES EH, HUDSON D, TINO WT, HUBER MJ, LAKE TP: **Development of human monoclonal antibodies specific for extracellular epitopes in prostate-specific membrane antigen (PSMA).** *12th Intl. Conf. Monoclonal Antibodies for Cancer* (1999) San Diego, CA, USA.
- Description of fully human monoclonal antibodies specific for protein conformational epitopes of PSMA.

32. VARKARAKIS MJ, MURPHY GP, NELSON CMK, CHEHVAL M, MOORE RH, FLOCKS RH: **Lymph node involvement in prostatic carcinoma.** *Urol. Clin. North Amer.* (1975) **2**:197-212.

33. KRAMER S, GORICH J, GOTTFRIED HW *et al.*: **Sensitivity of computed tomography in detecting local recurrence of prostatic carcinoma following radical prostatectomy.** *Br. J. Radiol.* (1997) **70**:995-999.

34. SANFORD E, GRZONKA R, HEAL A *et al.*: **Prostate cancer imaging with a new monoclonal antibody :a preliminary report.** *Annals Surg. Oncol.* (1994) **1**:400-404.

35. MURPHY GP: **Radioscintiscanning of prostate cancer.** *Cancer* (1995) **75**:1819-1822.

36. KAHN D, WILLIAMS RD, MANYAK MJ *et al.*: **Indium-111 capromab pendetide in the evaluation of patients with residual or recurrent prostate cancer after radical prostatectomy.** *J. Urol.* (1998) **159**:2041-2047.

37. KAHN D, WILLIAMS RD, SELDIN DW *et al.*: **Radioimmunoscintigraphy with $^{111}$indium-labeled CYT-356 for**

**the detection of occult prostate cancer recurrence.** *J. Urol.* (1994) **152**:1490-1495.

38. BABAIAN RJ, SAYER J, PODOLOFF DA *et al.*: **Radioimmunoscintigraphy of pelvic lymph nodes with $^{111}$Indium-labeled monoclonal antibody CYT-356.** *J. Urol.* (1994) **152**:1952-1955.

39. HASEMAN MK, REED NL, ROSENTHAL SA: **Monoclonal antibody imaging of occult prostate cancer in patients with elevated prostate-specific antigen: Positron emission tomography and biopsy correlation.** *Clin. Nucl. Med.* (1996) **21**:703-713.

40. ULCHAKER J, KLEIN E, ZIPPE C *et al.*: **Indium-111 capromab monoclonal antibody: Utility in differentiating local *versus* distant relapse after radical prostatectomy.** *J. Urol.* (1997) **157**(Abst):205 .

41. BURGERS JK, HINKLE GH, HASEMAN MK: **Monoclonal antibody imaging of recurrent and metastatic prostate cancer.** *Semin. Urol.* (1995) **13**:103-112.

42. KAHN D, WILLIAMS RD, HASEMAN MK, REED NL, MILLER SJ, GERSTBREIN J: **Radioimmunoscintigraphy with In-111-labeled capromab pendetide predicts prostate cancer response to salvage radiotherapy after failed radical prostatectomy.** *J. Clin. Oncol.* (1998) **16**:284-289.

43. POLASCIK TJ, GURGANUS RT, PARTIN AW *et al.*: **Comparison of staging algorithms and a monoclonal antibody scan (ProstaScint) to predict lymph node involvement in high-risk prostate cancer.** *J. Urol.* (1998) **159**:289.

44. CHENGAZI VU, FENELEY MR, ELLISON D *et al.*: **Imaging prostate cancer with technetium-99m-7E11.C5.3 (CYT-351).** *J. Nucl. Med.* (1997) **38**:675-682.

45. HINKLE GH, BURGERS JK, NEAL CE *et al.*: **Multicenter radioimmunoscintigraphic evaluation of patients with prostate carcinoma using indium-111 capromab pendetide.** *Cancer* (1998) **83**:739-747.

46. ELGAMAL AA, TROYCHAK MJ, MURPHY GP: **ProstaScint may enhance identification of prostate cancer recurrence after prostatectomy, radiation, or hormone therapy: Analysis of 136 scans of 100 patients.** *Prostate* (1998) **37**:261-269.

47. MANYAK MJ, HINKLE GH, OLSEN JO *et al.*: **Immunoscintigraphy with indium-111-capromab pendetide: Evaluation before definitive therapy in patients with prostate cancer.** *Urology* (1999) **54**:1058-1063.

48. MURPHY GP, MAGUIRE RT, ROGERS B *et al.*: **Comparison of serum PSMA, PSA levels with results of cytogen-356 ProstaScint scanning in prostatic cancer patients.** *Prostate* (1997) **33**:281-285.

49. FIGG WD, AMMERMAN BA, PATRONAS N *et al.*: **Lack of correlation between prostate-specific antigen and the presence of measurable soft tissue metastases in hormone-refractory prostate cancer.** *Cancer Invest.* (1996) **14**:513-517.

50. MURPHY GP, TROYCHAK MJ, COBB OE *et al.*: **Evaluation of PSA, free PSA, PSMA and total and bone alkaline phosphatase levels compared to bone scans in the management of patients with metastatic prostate cancer.** *Prostate* (1997) **33**:141-146.

51. DEB N, GORIS M, TRISLER K *et al.*: **Treatment of hormone-refractory prostate cancer with $^{90}$Y-CYT-356 monoclonal antibody.** *Clin. Cancer Res.* (1996) **2**:1289-1297.

52. HAMILTON A, KING S, LIU H, MOY P, BANDER N, CARR F: **A novel humanised antibody against prostate-specific membrane antigen (PSMA) for *in vivo* targeting and therapy.** *Proc. Amer. Assoc. Cancer Res.* (1998) **39**:440.

53. BANDER NH, NANUS D, BREMER S *et al.*: **Phase I clinical trial targeting a monoclonal antibody (mab) to the extracellular domain of prostate specific membrane antigen ($PSMA_{ext}$) in patients with hormone-independent prostate cancer.** *Proc. Amer. Soc. Clin. Oncol.* (2000) **19**:477a.

54. BANDER NH: **Monoclonal antibody therapy for prostate cancer.** *7th Annual CaP CURE Scientific Retreat*, Lake Tahoe, NV. (2000) www.capcure.org
- Results of a Phase I clinical trial with a PSMA-specific linear sequence epitope antibody.

55. SALGALLER ML, THURNHER M, BARTSCH G, BOYNTON AL, MURPHY GP: **Report from the International Union Against Cancer (UICC) Tumor Biology Committee: UICC workshop on the use of dendritic cells in cancer clinical trials.** *Cancer* (1999) **6**:2674-2683.

56. MURPHY GP, TJOA BA, SIMMONS SJ *et al.*: **Phase II prostate cancer vaccine trial: Report of a study involving 37 patients with disease recurrence following primary treatment.** *Prostate* (1999) **39**:54-59.

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